PREPARATION OF POLY(LACTIC ACID) (PLA) MICROSPHERES FOR DRUG DELIVERY SYSTEM

by

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Thesis submitted in fulfilment of the requirements

for the degree of Master of Science

May 2015

ACKNOWLEDGEMENTS

I would like to take this opportunity to show my greatest gratitude to all who assisted and helped me, directly or indirectly in my Master project. First and foremost, I would like to give my sincere thanks to Dr. Zuratul Ain Abdul Hamid, to be my supportive supervisor and guided me throughout the project. She always willingly shared the knowledge, valuable experiences and advises. Sincere thanks were also accorded to my co-supervisors, Prof. Hanafi Ismail and Assoc. Prof. Dr. Zulkifli Ahmad. Their supervision and supports were truly helped the progression and smoothness of this project.

Great deals appreciated goes to the technical staffs for the smoothness and co-operation by allowed me to hand-on the equipment and tools in the laboratory. I appreciated the valuable information and knowledge that shared by all staff in SMMRE for being patient in helping me. This project cannot be successfully implemented without the effective coordination done by all the involving staffs and lectures.

Lastly, I would like to thank my family and friends for their support which given me the strength to carry on when faced the difficulties during conducting in the works. Without their direct support, I might not able to complete this project successfully.

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LIST OF SYMBOLS

d_{max}	Maximum stable droplet Size
d_{32}	Sauter's mean diameter
D	Diameter of stirrer
ρ_c	Density of Continuous Phase
N	Stirring Speed
We	Weber Number
C_1	Coefficient of the correlation
C_2	Coefficient of the correlation
σ	Interfacial tension
μ_d	Viscosity of dispersed phase
μ_c	Viscosity of continuous phase
ϕ_d	Dispersed phase volume ratio
τ	Shear stress
μ	Eddy viscosity
γ	Shear rate
α	Coefficient that depend on many other factor
C_3	Coefficient of the correlation
C_4	Coefficient of the correlation
τ	Shear Stress
μ	Eddy Viscosity
γ	Shear Rate
ϕ_d	Volume Ratio of Dispersed Phase

$+ ve$	Positive
$- ve$	Negative
γ_{sv}	Interfacial energy between solid surface (*s*) and vapor (*v*)
γ_{sl}	Interfacial energy of solid and liquid (*l*)
γ_{lv}	Interfacial energy of liquid (*l*) and vapor (*v*)
θ	Water contact angle
γ_L^d	Dispersion forces of liquid surface tension
γ_L^P	Polar forces of liquid surface tension
A_1	Absorbance of first sample
A_2	Absorbance of the second sample
A_n	Absorbance of the *n* th sample
n	The number of sample
R	The extracted volume
W	The total weight of the model drugs
m	Slope of the graph
C	y-intercept of graph

LIST OF ABBREVIATIONS

PLA	Poly(lactic acid)
ESE	Emulsion and solvent evaporation
PSD	Particle size distribution
HCl	Hydrochloride acid
NaOH	Sodium hydroxide
DP	Dispersed phase
CP	Continuous phase
T	Turbulence flow
V	Viscous forces in laminar flow
C	Cavitation
L	Low
M	Moderate
H	High
B	Batch
C	Continuous
V	Viscous
N	Not so viscous
W	Aqueous
DPV	Dispersed phase volume
PLLA	Poly(L-lactic acid)
PLGA	Poly(lactic-co-glycolic)
PDLLA	Poly(D,L-lactic acid)
DCM	Dichloromethane

PVA	Poly(vinyl alcohol)
RhB	Rhodamine B
MO	Methyl Orange
SEM	Scanning electron microscopy
DCAT	Dynamic contact angle and tensiometer
UV-Vis	Ultraviolet-visible
XPS	X-ray photospectroscopy
DSC	Differential scanning calorimetry
Log P	Partition coefficient

PENYEDIAAN MIKROSFERA POLI(ASID LAKTIK) (PLA) UNTUK SISTEM PENGHANTARAN UBAT

ABSTRAK

Poli(asid laktik) (PLA) mikrosfera telah berjaya disediakan melalui kaedah emulsi dan pemeruapan pelarut (ESE). Taburan saiz partikel (PSD) mikrosfera yang diperoleh adalah dalam lingkungan 1 - 250 μm, merupakan lingkungan saiz yang boleh diterima bagi penghantaran jenis suntikan "parenteral". Kaedah "shake flask" dibuktikan sebagai teknik alternatif untuk menentukan kebolehlaksanaan pengkapsulan sesuatu ESE sistem. Dalam projek ini, pasangan pelarut diklorometana-air telah digunakan dalam sistem shake flask untuk mensimulasikan sistem ESE, dan Rhodamine B telah digunakan sebagai model ubat sepanjang projek ini. Parameter yang dikaji dalam fabrikasi mikrosfera adalah kepekatan PLA, kepekatan PVA dan nisbah isipadu fasa teragih (DP). Kesan parameter pada PSD, morfologi permukaan dan kecekapan pengkapsulan telah dinilai. Formulasi optimun adalah pada 25% DP, 15% PLA dan 1% PVA, yang mana hasil mikrosfera tertinggi diperolehi dengan penggunaan PVA terendah dalam ESE sistem dan taburan partikel saiz diperolehi dalam 1- 50 μm. Bagi formulasi ini, pembusaan emulsi telah diperhatikan semasa pengacauan dalam proses pengemulsian. Dicadangkan bahawa, titisan yang tersebar dalam emulsi lebih cenderung berada dalam struktur buih yang seterusnya memberikan kestabilan tambahan antara titisan atau partikel separuh keras tersebut. Teknik hidrolisis pengaruh pemangkin telah digunakan untuk mengubahsuaian permukaan mikrosfera. Kajian atas sifat-sifat permukaan dan pukal telah dijalankan untuk mengesahkan keberkesanan teknik hidrolisis. Secara keseluruhan, faktor-faktor termasuk skala masa, jenis pemangkin dan kepekatan pemangkin harus dimanipulasi untuk mendapatkan sifat permukaan yang dikehendaki (contohnya, hidrofilik, caj dan morfologi permukaan) dengan ubah bentuk sifat pukal mikrosfera yang minimum.

PREPARATION OF POLY(LACTIC ACID) (PLA) MICROSPHERES FOR DRUG DELIVERY SYSTEM

ABSTRACT

Poly(lactic acid) (PLA) microspheres were fabricated through emulsion and solvent evaporation (ESE) technique. The particle size distributions (PSD) of microspheres obtained were in the range of 1- 250 µm, as within the range of acceptable size in parenteral injection. A shake flask method was demonstrated as an alternative technique to determine the encapsulation feasibility of an ESE system. Herein, dichloromethane-water solvent pair was used in shake flask system in order to simulate ESE system, and Rhodamine B was utilized as model drug throughout the project. In the fabrication of microspheres, the studies parameters were included PLA concentration, PVA concentration and dispersed phase volume ratio (DP). The effect of parameters on PSD range, surface morphology and encapsulation efficiency were evaluated. The optimum formulation was at 25 % DP, 15 % PLA and 1 % PVA, wherein the highest output of microspheres obtained at lowest PVA consumption in the system and narrow PSD obtained in range 1- 50 µm. In this formulation, extensive foaming of emulsion was observed during the emulsification process. It was suggested that the dispersed droplets tends to stay within the foam structure which further provide extra stabilization between droplets or partial solidified particles. The surfaces of microspheres were modified through catalytic induced hydrolysis technique. The surface and bulk properties of treated microspheres were investigated to verify the effectiveness of hydrolysis technique. Results demonstrated that the factors included timescale, type of catalyst and concentration should be manipulated in order to obtain the desired surface properties (e.g. hydrophilicity, surface charges and surface morphology) with minium deformation in microspheres bulk propertie

Chapter 1

Introduction

1.1 Overview

Drug delivery system is defined as the device which encapsulated with specific amount of drug further delivery it in a controlled or sustained manner upon the administration. In pharmaceutical, it is urged to have the suitable drug delivery system that able to maintain the blood drug concentration lies between the minimum effective and maximum toxic concentration (Uchizono, 2006, Liechty *et al*., 2010). In order to deliver drugs to diseased site in the body in a more effective and less invasive way, a new dosage form technology of drug delivery has emerged in the late 1960's (Coelho *et al.*, 2010).

There are variable approached in designing an effective drug delivery system by using polymeric materials either in natural or synthetic forms. One of the promising design is used of biodegradable microspheres prepared from polyester, such as poly(lactic acid) (PLA), poly(glycolic acid) and their copolymer poly(lactic-co-glycolic). They are mostly focusing on the encapsulation of large molecules, e.g., peptides, proteins and plasmid Deoxyribo Nucleic Acid (DNA) for potential used as vaccines or as long-acting release drug formulation. The major advantages of biodegradable systems are they was eventually absorbed or excreted by the body. The drug release of microspheres was controlled by the polymer degradation rate and particle size of microspheres (Ulery *et al.*, 2011). One of the major considerations in microspheres fabrication was the particle size distribution as it determined the drug administration route, drug release properties, patient compliance and safety during the application period (Berkland *et al.*, 2002).

Drug loaded microspheres are prepared through microencapsulation technique. Microencapsulation, is the term used to describe the technique to surround or coat certain chemical entity (in the form of either solids, liquids or gaseous) within a material that able to release the contents at certain conditions (upon moisture, pH, physical force or combination thereof) (Luzzi, 1970, Chanana *et al.*, 2013). It has been reported that emulsion and solvent evaporation technique is most successful to load either insoluble or poorly soluble drugs in biodegradable microspheres (O'Donnell and McGinity, 1997). The effectiveness of microspheres preparation technique for drug delivery usage is depends on the successful entrapment of the drug within the particles. The understanding ESE technique from the aspect of intrinsic (interaction between materials) and extrinsic (adjustable parameters such as stirring speed, water-in-oil phase ratio, drug loading, polymer and stabilizer concentration) are crucial in order to produce microspheres with desired drug encapsulation.

Another consideration involved in designing an effective drug delivery system is the surface properties of microspheres. Generally, the surfaces of polyester based biodegradable microspheres are recognized as non-biocompatible because of hydrophobic in nature and lacking of bio-recognized functional group or molecules. These might cause rapid elimination of microspheres in the biological system prior drug release from microspheres (Stolnik *et al.*, 1995). Material surfaces can be modified by a variety of different methods, such as the application of a surface chemical gradient, self-assembled films, surface-active bulk additive and surface chemical reaction. Microspheres surfaces are required to be modified with various functional groups such as methyl-, hydroxyl-, amino-, and carboxyl, all of which can be found on natural biological surfaces in order to optimize the function of microspheres as drug delivery system (Roach *et al.*, 2007).

1.2 Problem Statements:

Emulsion and solvent evaporation (ESE) was selected as microspheres fabrication technique, because this technique is conceptually simple in experimental setup. However, the quality of microspheres (e.g. particle size, drug loading, agglomerate and etc.) prepared from this technique are highly dependent on the system nature. Despite the widespread use of this technique, encapsulation methodologies are still largely based on trial and error. More quantitative theory and experiments are required to improve our understanding of how the encapsulation conditions affect the final particle characteristics. Therefore, at the preliminary stage of microsphere fabrication the establishment and understanding of ESE technique are required.

The encapsulation feasibility of the ESE process is highly dependent on the materials interaction and the materials' properties. As illustrated in Fig. 1.1, the feasibility of the system to encapsulate certain chemical substance in PLA microspheres can only be determined if the series of ESE process was run and the obtained microspheres were characterized. Therefore, an alternative approaches was proposed to determine the encapsulation feasibility to eliminate this trial-and-error procedure. In this research, a shake flask method was proposed to tackle this issue. In this shake flask approach, if the drug has higher affinity toward the polymer's solvent in shake flask system, then this drug is able to be encapsulating within the microspheres in through ESE technique.

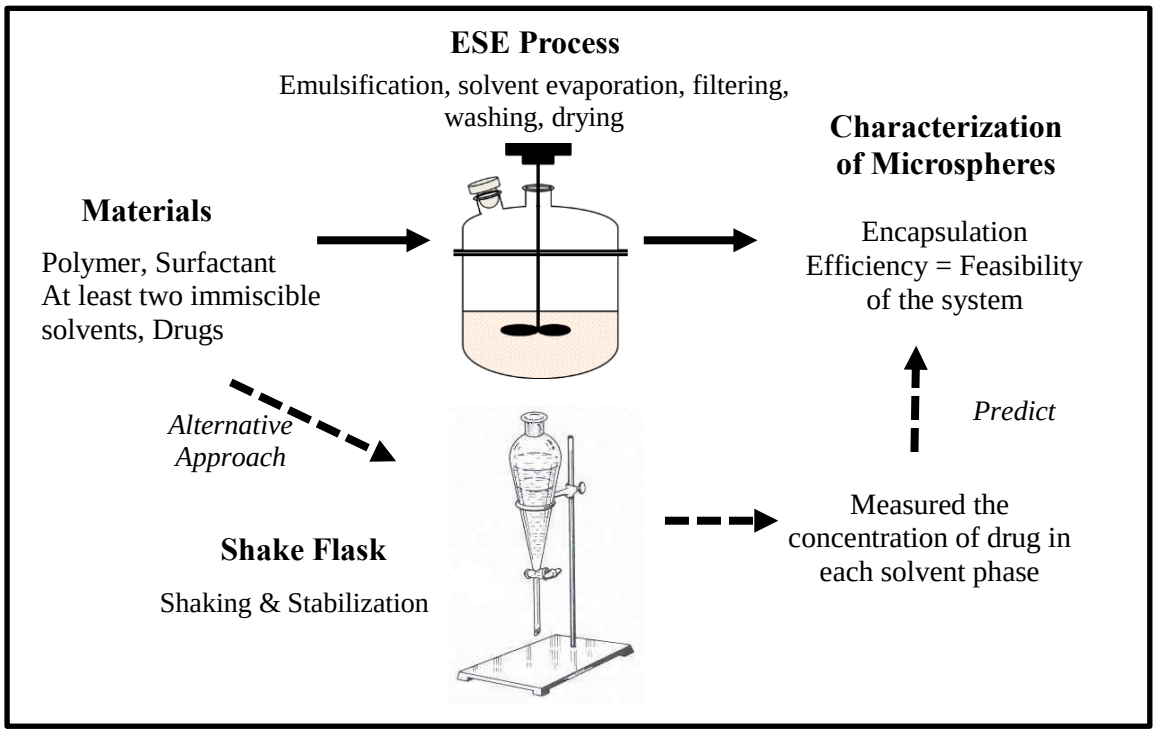


Fig. 1.1: Shake flask method proposed to replace the ESE processes during the determination of encapsulation feasibility

Particle size distribution of microspheres is crucial in drug delivery purposes. Unfortunately, the main issue of using the ESE technique in microspheres fabrication was the broad particle size distribution. Various approaches have been conducted to narrow the PSD, through either high stirring forces by homogenizer and sonication, or through highly controllable electrohydrodynamic atomization and membrane emulsification (Liu *et al.*, 2011, Vauthier and Bouchemal, 2009, Pareta and Edirisinghe, 2006, Sawalha *et al.*, 2008). However, the mentioned modified techniques are required extra machinery setup or exposed the risk of emulsion heat up that induces the deterioration of drug (Freitas *et al.*, 2005). Therefore, it is desired to improve the ESE system which able to narrow the particle size distribution without possessing any difficulty in either the fabrication process or drug deterioration.

As polyester polymer are lacking active functional group to bind biomolecules, the stealth molecules are generally adsorbed on microspheres surface as coating to either improve the hydrophilicity property or introduce bio-recognized functionality (Gref *et al.*, 2012). However, the stealth coating formed through adsorption was unstable as it might easily desorbed if the environment condition was changed or varied. Therefore, it is desired that the surface functionalization of microspheres can be conducted in order to introduce active functional group and further enable the covalently crosslink with any biomolecules. Through the reviewing, it was noticed that microspheres are rarely modified through the conventional surface modification techniques, such as hydrolysis, ultraviolet photo-grafting, plasma radiation, and electron beam radiation (Slepička *et al.*, 2013). In spite of that, it is worth exploring the potential of those conventional techniques as they able to diversify the surface's functionality of microspheres. In this research, catalytic induced hydrolysis is selected to modify microspheres surfaces. However, it is generally known that hydrolysis is the major degradation mechanism of polyester. Therefore, the effect of hydrolysis on either improving the surface properties or deforming the bulk properties are required to be studied.

1.3 Objectives:

1. To establish the microspheres fabrication processes through the understanding of influencing factors in the processes.
2. To determine the effectiveness of shake flask method to serve as alternative approach in predict the encapsulation feasibility of microspheres.
3. To investigate the properties of microspheres prepared by different formulations through emulsion and solvent evaporation technique.
4. To investigate the effect of catalytic induced hydrolysis in microspheres surface modification.

1.4 Research Organization

The research first stage involved preliminary studies, in the determination of materials' concentration and the polymer solutions' properties (e.g. viscosity, interfacial tension, surfactant adsorption). Shake flask method was used to select a suitable model drugs for PLA microspheres. The second stage of the research involved the fabrication of PLA microspheres at the factors of PLA concentration, PVA concentration, and dispersed phase volume fraction. Single emulsion was used to prepare unloaded PLA microspheres, while double emulsion was used to prepared model drug encapsulated microspheres. Microspheres properties were studied on the aspect of particle size distribution, surface morphology, encapsulation and loading efficiency. The release of model drug was conducted in condition of 37˚C and catalyzed accelerated medium. Lastly, the microspheres were surface modified through catalytic induced hydrolysis method, where the catalysts used are hydrochloride acid and sodium hydroxide. The surface and bulk properties of surface treated were comprehensively studied, to validate the potential of hydrolysis method in modified microsphere's surfaces.

Chapter 2

Literature Review

2.1. Introduction

Controlled drug delivery technology represents one of the frontier areas of science in biopharmaceutical and biomaterials applications (Singh *et al*., 2010). This new dosage form technology of DDS has emerged in the late 1960's in using polymers (Coelho *et al.*, 2010). The idea of DDS is aimed to maximize efficiency of the current available drugs, and further delivery drugs to diseased site in the body it in a more effective and less invasive way.

By definition, drug is the term used to describe a chemical substance that able to treat certain illness. The therapeutic effect of drug usage is dependent on its concentration in blood plasma. As shown in Fig. 2.1, the effectiveness of drug is laid within a therapeutic window where above the window limit will lead to toxicology effect, and below the window limit will loss in therapeutic effect.

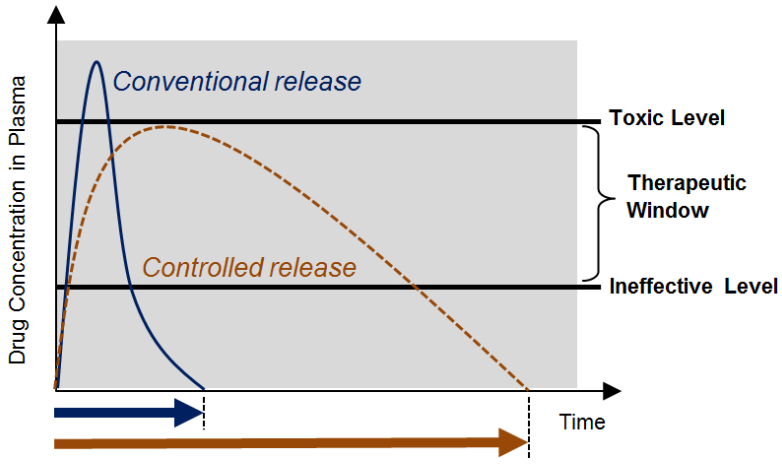


Fig. 2.1: Theoretical plasma concentration in conventional and controlled release drug delivery

In conventional drug delivery (e.g. oral pills and parenteral injection), the drug concentration in blood plasma is rapidly increased beyond upper limit and decreases below lower limit of therapeutic window. The repeated uptake of drug is required at typical every 8 hours or 24 hours along the treatment to ensure the drug concentration reached within therapeutic window (Shaik *et al*., 2012). A controlled DDS possessed advantages in maintained the drug concentration within the therapeutic window and sustained for longer period of time. The prolong drug release is able to reduce the drug administration frequency, and also reduced the risk of toxic effect.

2.2 Polymeric Microspheres

Microspheres are characterized as the powders prepared from either nature or synthetic polymer having a particle size range from 1-1000 μm (Sahil *et al*., 2011). They have been routinely used in the biomedical and bioprocess applications. For example, polystyrene microspheres have been used in high performance liquid chromatography (Kirkland *et al.*, 2000). In flow cytometry procedure, polystyrene microspheres with the encapsulation of fluorescent substances are typically used to calibrate the absolute number of specific lymphocytic cluster of differentiation cell (Zhang *et al.*, 2009). Polyethylene microspheres are used as permanent or temporary fillers (Khan *et al.*, 2012). Biodegradable microspheres have also been used a bulking agents in soft tissue to augment the efficiency of opening-closing system, which included in treatment of stress urinary incontinence. Furthermore, in embolization therapies which purpose to occlude blood vessel in assist surgical operation (Saralidze *et al*., 2010).

2.2.1 Biodegradable Microspheres

Biodegradable microspheres are generally acting as a drug delivery depot which also known as drug cargo. This type of drug delivery depot is usually formulated peptides or proteins in order to achieve sustain delivery that usually up to 2 - 3 years. They performed drug release along with the degradation of polymers and no further surgical removal is required after treatment completed. The long term degradation properties of PLA giving great opportunities in developing sustained and controlled DDS (Shaik *et al*., 2012).

The available polymers that exhibited both biodegradable and biocompatible properties have included poly(lactic acid), poly(glycolic acid), polyhydroxyalkanoates, poly(caprolactone), poly(propylene fumarate), polyanhydrides, poly(other esters) and etc. Review done by Ulery *et. al* (2011), indicated that PLA are still the most potential candidates for drug delivery purposes. The predominate advantages of PLA is that there are commercially available and reasonable degradation rate (with degradation rate constant of 6.6×10^{-6} s^{-1}). They also have largely available toxicological and chemical data, predictable biodegradation kinetics, ease of fabrication, versatility in properties, variety in copolymers ratios and molecular weights, regulatory approval (Jain, 2000, Sosnowski, 2001, Vroman and Tighzert, 2009, Nampoothiri *et al*., 2010, Ulery *et al.*, 2011).

2.2.2 Type of Microspheres

Microspheres are sometimes known as microcapsules, which are differential from their structural design in drug encapsulation as illustrated in Fig. 2.2. “Microcapsules” is used to describe the particles which contained a core (solid or liquid drug) which coated by a polymeric material (either single or multilayers). They also known as reservoir devices disregard to the size of particulates. While “microspheres” is used to describe the monolithic type device where the drugs molecules are dissolute or dispersed within the particles’ matrices (Obeidat, 2009).

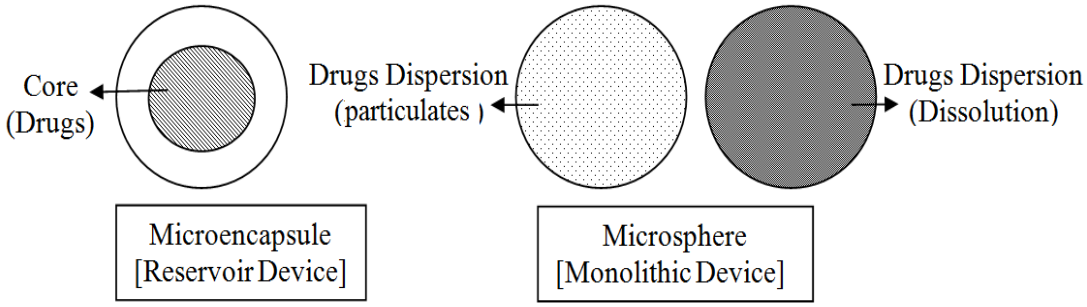


Fig.2.2: Schematic of the microcapsule (reservoir device, left) and microsphere (monolithic device, right)

2.2.3 Particle Size of Microspheres

The common drug delivery route of microspheres was through parenteral and oral administration. Particle size and distribution range is one of the critical properties, as it greatly affected the patient compliance and safety during the application period. The particular considerations on microspheres’ size have been summarized and listed in Table 2.1.

Table 2.1: Desirable particle size of microspheres at different administrative route and consideration

Administrative Route & Particle Size		Considerations
Parenteral Injection (Intravenous, intramuscular , subcutaneous)	< 250 µm, *ideally* < 125 µm	Normal needle's size are 22 -25 gauges (inner diameter of 394 µm-241 µm). Patient compliance improved as needle size decreased. Microspheres size determined the needle size and the risk of needle blockage. (Ye *et al*., 2010)
	10 - 250 µm	Minimize the microphages phagocytosis and inflammatory reaction (Tabata and Ikada, 1990)
	< 100 µm	In the case of specific organs such as the brain, microspheres size should not exceed 100 µm so as not to disturb the 3D structure of the brain (Tran *et al.*, 2011)
	10 - 120 µm	Suitable drug depot's size for subcutaneous or intramuscular delivery (Berkland *et al*., 2004)
Oral Administration	7.2 - 2.1 µm	Induction of mucosal immunity by particles from 5 -10 µm. Induction of systemic and local immune responses by particles < 5 um (Kofler *et al*., 1996)
	Around 3 µm	Pulmonary route, microspheres should be around 3 µm
	< 5 µm	Particles between 3 -10 µm tends to retain in the Peyer's patches of gut associated lymphoid tissue after oral administration. Whereas < 5 µm ease to across the gastro-intestinal (GI) tract and are progressively distributed to other sites (Yeh *et al*., 1995)
Preoperative embolization	100-160 µm	In order to reach the precapillary arterioles (30- 160 µm) without passing through the capillary filter (10 -30 µm), microspheres size should be in range 100-160 µm. < 50 µm would be retrieved in the general circulation, and preferentially stopped in pulmonary vessels where they may cause adverse effects resulting either in mechanical obstruction or even in direct injury to the lung itself (Grandfils *et al*., 1992)

2.3 Microspheres Fabrication

Microencapsulation is the term used to describe the technique used to wrap the chemical entities within an individual or protective coating (Chanana *et al.*, 2013). A number of microencapsulation techniques have been developed included, emulsion and solvent evaporation/extraction, spray drying, phase separation (coacervation), atomization and etc. (Jain, 2000, Reis *et al*., 2006). Amongst those, emulsion and solvent evaporation (ESE) is still being widely employed in polymeric microspheres fabrication.

ESE technique consists of two major processes, which is emulsification and solvent evaporation/removal. The flow chart in Fig. 2.3, illustrated the sequence of particles formation through an ESE processes (O'Donnell and McGinity, 1997). In this method, the pre-formed polymer is used and dissolved into it particular solvent. The drug solution or solid particle are firstly dispersed in the polymer solution, and then emulsified in a surfactant containing continuous solution. After droplets formation, the process is followed by solvent diffusion and evaporation from micro-droplets leading to particle formation (Bodmeier and McGinity, 1987, Astete and Sabliov, 2006).

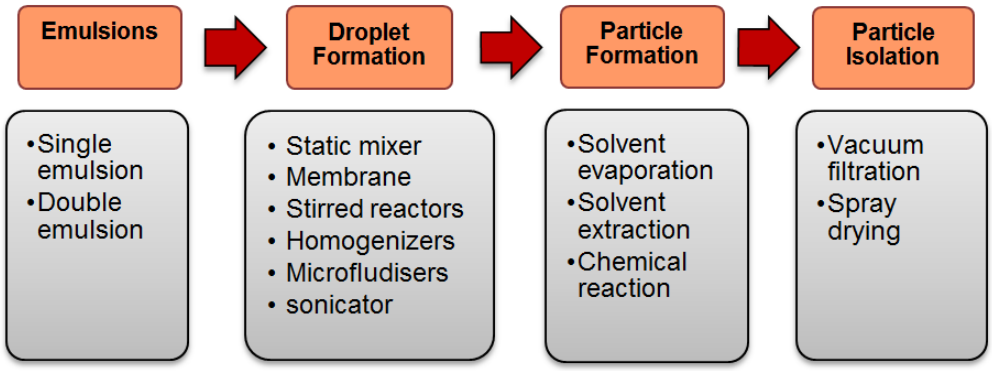


Fig. 2.3: Standard process in synthesis PLA microspheres by emulsion and solvent evaporation technique with different process options at each stage

2.3.1 Material Selection

In designing new potential drug encapsulated microspheres through ESE system, the considerations included are (i) the desired drugs and their chemical properties, (ii) polymer matrices and their chemical properties, (iii) type of emulsion system, and lastly (iv)the fabrication parameters and conditions. The encapsulation feasibility of the ESE process is measured from microspheres obtained and known as encapsulation efficiency. From the best of author's knowledge, there are no alternative techniques to determine the encapsulation feasibility of an ESE system, except running throughout the system.

Numerous papers have summarized the dependency of materials usage and process parameters in affecting the encapsulation efficiency (Jyothi *et al*., 2010, Dhakar *et al*., 2012, Prabu *et al.*, 2009, Dupinder and Seema, 2013, Dhakar *et al*., 2010). ESE is a simple technique without the involvement of complicated reactions. Yet, it is very dependent on the nature of emulsion which comprised of various polymers, surfactant, drugs and solvents. The materials' interaction has been studied from the aspects of interaction energy, mutual solubility and the compatibility (Liu *et al.*, 2004, Gander *et al.*, 1995, Matsumoto *et al*., 2008). Gander *et. al* (1995) have reported that the high interaction energies between drug-polymer-solvent is significantly reduce the encapsulation efficiency of bovine serum albumin in poly(lactide) microspheres. The mutual solubility acetone-water-glycerol reported in ternary phase diagram by Matsumoto *et. al* (2008) have showed to affect the formation poly(lactic-co-gylcolic) microspheres and encapsulation efficiency. While Liu *et. al* (2004) have highlighted polymer-drug compatibility from the aspect of enthalpy of mixing and partial solubility parameters, in determine the suitable polymer to encapsulate Ellipticine.

In this project, an alternative technique is being proposed to determine the encapsulation feasibility of ESE without having a series of fabrication processes. The alternative technique is known as “shake flask” as illustrated in Fig. 2.4. It is hypothesized that, if the drug molecules have higher affinity to distribute in polymer’s solvent the ESE process will be feasible to encapsulate the drug.

In general, shake flask method being used in laboratory extraction or purification process, and also as a laboratory test in determination of partition coefficient (*Log P*) of the chemical entity. The immiscible biphasic liquids are consisted of hydrocarbon-aqueous pair, typically octanol-water pair since they have great difference in polarity (Sangster, 1989). Shake flask have few similarity with emulsion, where both of the system involved (i) two immiscible solvents, (ii) an extractable chemical substance and (iii) create large interfacial area during stirring or shaking process.

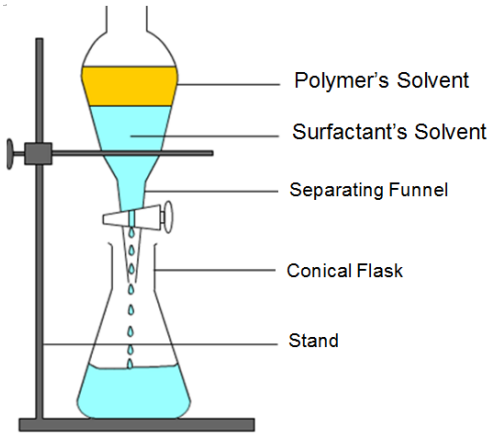


Fig. 2.4: The schematic illustration of shake flask

2.3.2 Making the Emulsion

Emulsion is a colloidal dispersion, where a liquid is dispersed in a continuous liquid phase at different composition (Tadros, 2013, Schrammand, 2005). The type of emulsion may be distinguished according to the nature of solvent which is aqueous (water) and organic (oil), and their sequence of emulsification, as illustration in Fig. 2.5.

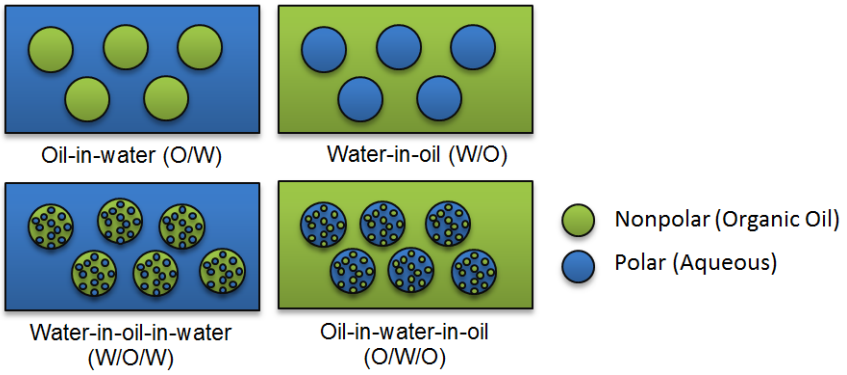


Fig.2.5: General type of (a) single and (b) double emulsion that able to be produced through ESE technique

A typical oil-in-water emulsion and description terms are showed in Fig. 2.6. Emulsification involved the breakage of liquid dispersed phase into dispersed droplets within a continuous phase. External forces are normally supplied in the emulsification, and these forces are sufficient to overcome the cohesive forces of liquid which allowed droplets formation. With the presence of surfactant, the droplets formed can be stabilized further solvent evaporation will led to particle formation.

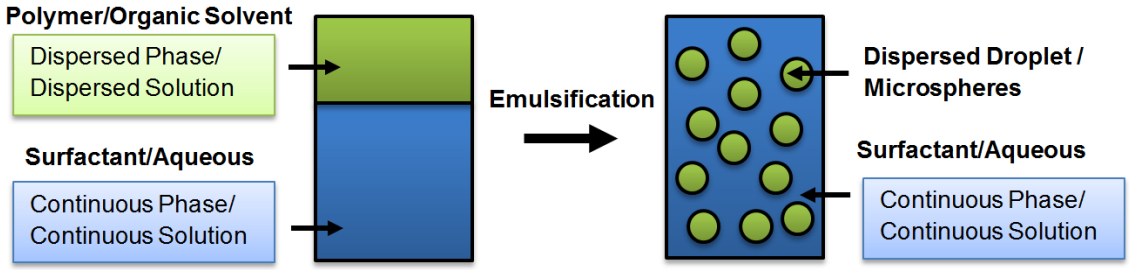


Fig. 2.6: Terms used in described an oil-in-water emulsion

2.3.2.1 Stirring of Emulsion

In emulsification, stirring is an important parameter which controlling the size of microspheres. Many other factors linked to stirring also have impact on the size of microspheres such as the geometry of the reactor, the number of impellers and their position, and the ratio of impeller's diameter compared to the reactor's diameter (Li *et al.*, 2008, Puel *et al.*, 2006). The common mechanical forces used in emulsification are

summarized in Table 2.2, included stirrer, homogenizer and sonicator (Walstra, 1983). Smaller droplet size is obtained from techniques that provide higher energy density. However, the solution mixture have higher tendency to be heated up and might affect the quality of drug.

Table 2.2: Technique of mechanical stirring to produce emulsion

Technique	Droplets Mainly Disrupted by [a]	Energy Density [b]	Mode of Operation [c]	Restrictions [d]
Stirrer	T, V	L	B	-
Homogenizer	T, C, V	H	C	N
Sonicator	C, T	M - H	C	W

[a] T= Turbulence, V= Viscous forces in laminar flow, C= Cavitation, [b] L= Low, M= Moderate, H= High, [c] D= Batch, C= Continuous, [d] The continuous phase should be, v= viscous, N= not so viscous, W=aqueous

The correlations that account the aspects of emulsion phases and factor linked to agitation in predict the droplet size is shown in Eq. (2.1) & (2.2) (Heiskanen *et al.*, 2012). As stirring speed increases, the higher shearing forces created will thus led to smaller particle size. As reported in others research works, the stirring speed is always a dominating factor to reduced particle size as it provide shearing energy to disperse the dispersed phase in continuous phase (Yang *et al.*, 2001).

$$\frac{d_{max}}{D} = c_1\left(\frac{\rho_c N^2 D^3}{\sigma}\right)^{-0.6} = c_1 We^{-0.6} \quad (2.1)$$

$$\frac{d_{32}}{D} = c_2 We^{-0.6} = c_2 d_{max} \quad (2.2)$$

Where the symbol denoted, d_{max} is the maximum stable droplet size, D is the diameter of stirrer, ρ_c is the density of continous phase, N is the stirring speed, We is the Weber Number, and lastly c_1 and c_2 are the coefficient of the correlation.

2.3.2.2 Viscosity of Solution

In droplets formation, the viscosity of solutions is an important factor determined the size of droplets. The correlation between microspheres size and solution viscosity is shown in Eq. (2.3) & (2.4) (Li et al., 2008, Maa and Hsu, 1996). At higher viscosity, the viscoelasticity forces of the solution tend to restrict the liquid breakage into droplets. The microspheres size increased exponentially as the dispersed phase viscosity increased.

$$d_{32} = A\,(\frac{\mu_d}{\mu_c})^{0.25} \tag{2.3}$$

$$\tau = \mu\,\frac{dv}{dl} = \mu\gamma \tag{2.4}$$

Where the symbols denoted; μ_d is the viscosity of DP, μ_c is the viscosity of CP, τ is the shearing forces (stress), μ is the eddy viscosity, γ is the shear rate and A is a coefficient that depend on many other factors.

In single emulsion system, the viscosity of continuous phase is desired to be higher as compared to dispersed phase. During the emulsification, the turbulent flow of continuous phase tends to produce the shearing force, acting on dispersed phase which encourage the liquid breakup into droplets. As the continuous phase's viscosity increases, the shearing force produced also increases which able to reduced droplets size. In an oil-in-water emulsion, the viscosity of continuous phase is generally lower than the viscosity of dispersed phase.

2.3.2.3 Emulsion Phase Volume Ratio

Another important aspect in the emulsification is the phase volume ratio, where the correlation of in Eq. (2.5). In general, the increased of dispersed phase volume ratio (ϕ_d) will tend to reduce the turbulent level of the emulsification system. This will indirectly

reduce the shearing force of the system that used in liquid breakage, and thus resulting larger droplets. Furthermore, at higher ϕ_d condition tends to induce higher collision rates of droplets as there are more droplets in the continuous phase, further encouraged the coalescence of droplets resulting in larger particle size of microspheres (Heiskanen *et al*., 2012).

$$\frac{d_{32}}{D} = c_3 (1 + c_4\phi_d) We^{-0.6} \quad (2.5)$$

Where ϕ_d is the dispersed phase volume ratio and c_3 is the coefficient of the correlation.

2.3.2.4 Interfacial Energy

During droplets formation, the total interfacial area of two immiscible liquids is significantly increased and followed by the interfacial energy. Fig. 2.7 illustrated the relation between droplets size, interfacial energy and interfacial area (Schrammand, 2005). In the excess of energy at the droplet's interfaces, the droplet is thermodynamically and the destabilization of emulsion is likely to occur. This emulsion destabilization tends to prepare microspheres in larger particle size. Thus, the interfacial energy has to be reduced in order to produce smaller particles.

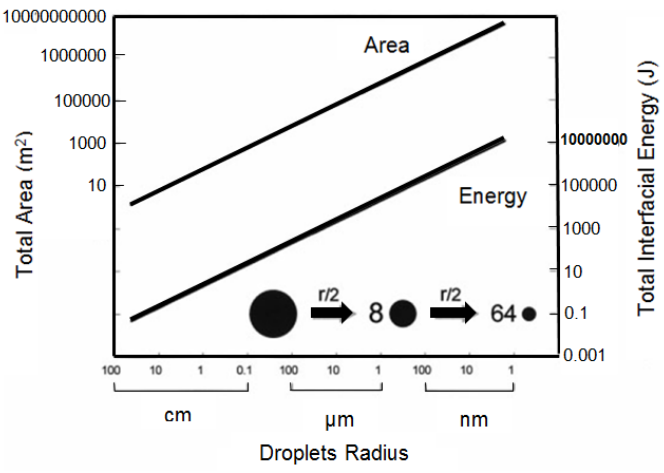


Fig. 2.7: Illustration of total area and energy changes involved in emulsifying one barrel of oil into water by dispersing into progressively finer droplets

One way to reduce the interfacial energy is by the addition of surfactant into the emulsion system. Since surfactant is able to interact at both dispersed phase and continuous phase, the adsorption of surfactant at the interfaces can reduces the interfacial energy. The presence of small amount of surfactant is able to lower the amount of energy required for emulsification by several orders of magnitude (Schrammand and Stasiuk, 2006). Furthermore, the adsorbed layers of surfactant on interfaces are able to acting as a structural physical barrier to against coalescence mechanism. In general, charged surfactant provides stabilization through electrostatic repulsion, while for non-ionic macromolecules provide stabilization through steric repulsion.

2.3.3 Stabilizing of Emulsion

The destabilization of droplets in an emulsion is contributed by various factors and the destabilization mechanisms are illustrated in Fig. 2.8.

- Fig. 2.8 (a) illustrated the destabilization caused by the density differences caused a total decay of the emulsion into two layers by either creaming or sedimentation process.
- Fig. 2.8 (b) illustrated the destabilization caused by the high attractive forces between droplets surfaces induced flocculation or agglomeration of the dispersed droplets.
- Fig. 2.8 (c) illustrated the collision of droplets which further induced the coalescence deformation and larger droplets are formed (Damodaran, 2006).
- Fig. 2.8 (d) illustrated destabilization by Ostwald ripening. It is a mechanism in minimizing the surface energy of the system where the small droplets tends to disappear caused by the dissolution in continuous medium, and fused with other droplet to form larger size droplet (Chistyakov, 2001, Filippini *et al.*, 2012).

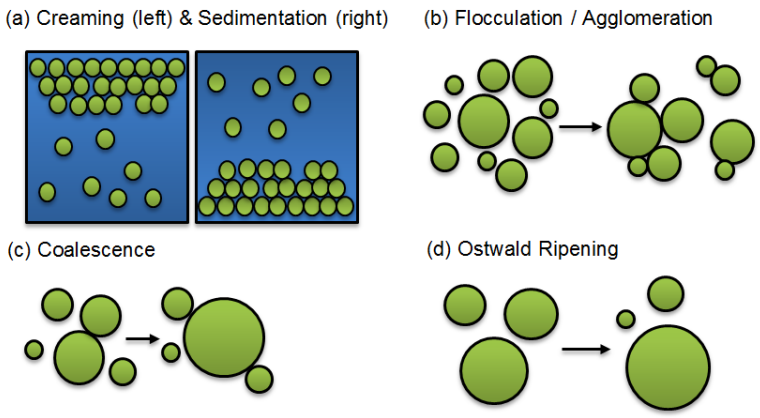


Fig. 2.8: Droplet mechanisms that lead to the deformation of an emulsion

Coalescence is a droplet deformation mechanism that involved the merging of two or more dispersed droplets to form a larger droplet. The coalescence rate is also depends on the number of droplet collisions, which dependent on the dispersed phase volume (DPV) in the system. DPV can also be interpreted in term of ϕ_d or droplets density in the emulsion system. Fig. 2.9 is an illustration regarding of the different DPV in constant CP volume. A dilute emulsion contained DPV in range 0.01 - 0.3 and typically produced particle spherical in shape. While a mild emulsion contained DPV in range 0.2 - 0.74, the mutual contact between dispersed droplets might leads to the formation of dense packing particles. As DPV achieved ≥ 0.9, a concentrated emulsion formed and the droplets would come into a close contact due to room limitation. This will then leading toward coalescence process and forming of larger particles (Chistyakov, 2001).

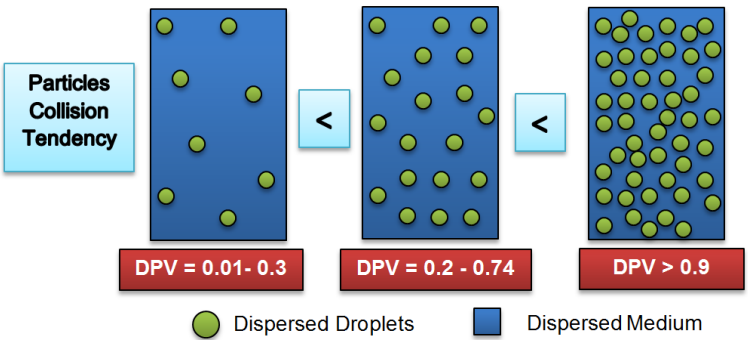


Fig. 2.9: Particles or droplets collision tendency a different DPV fraction

2.3.4 Particle Formation

Microspheres are obtained from the precipitation of emulsified droplets. As illustrated in Fig. 2.10, there are two main mass flows involved in droplets precipitation; solvent diffused from droplets into CP (solvent extraction) and solvents in CP diffused and evaporated into the air (solvent evaporation). Along the two mass flows, the droplets become rich in polymer and less in solvent, followed by precipitation of microspheres (Hwisa *et al*., 2013).

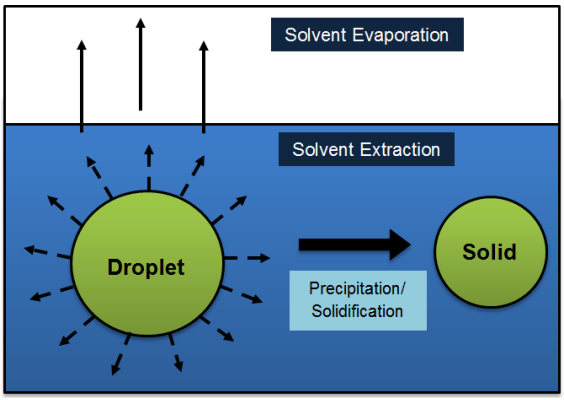


Fig. 2.10: Illustration of solvent extraction and evaporation in droplets solidification

2.4 Factors Influence Microspheres Properties

The factors which affecting the microspheres properties in ESE technique are listed in Table 2.3 (Freitas *et al*., 2005, Li *et al*., 2008). For materials involved in dispersed phase, the polymer is used as microsphere's matrix and the soluble solvent of the polymer. The drug is incorporated into the polymer solution in either liquid or solid form. While surfactant in continuous phase is function to stabilize the droplet formed in the emulsion. The usage of materials in term of concentration and volume fraction is greatly affected the microspheres properties.

Table 2.3: Factors that influence the microspheres properties in ESE technique

Factors in Emulsion and Solvent Evaporation		
Material Properties	Parameters	Operating Conditions
Dispersed Phase Polymer Solvent/ co-solvent Drug **Continuous Phase** Surfactant	Viscosity of DP and CP Volume fraction of DP:CP Drug Concentration in DP Concentration of polymer and surfactant	Geometry of reactor and agitator Agitation rate Temperature Pressure

The studies of this project does not included the factors involved in operating conditions as those factors are much dependent of the laboratory equipment. The materials properties and the parameters which affecting the solution properties are being studied. In this project, the polymer and it soluble solvent are poly(lactic acid) and dichloromethane respectively. While, poly(vinyl alcohol) is selected as the surfactant.

2.4.1 Poly (lactic acid) (PLA)

PLA is a polymer prepared from lactic acid (2-hydroxyl propionic acid). The usages of PLA in medical applications have been widely accepted due to their bioresorbable and biocompatible properties (Auras *et al.*, 2004). PLA is versatile to be fit in various applications as it exists in two optical isomers -D and -L, as illustrated in Fig. 2.11.

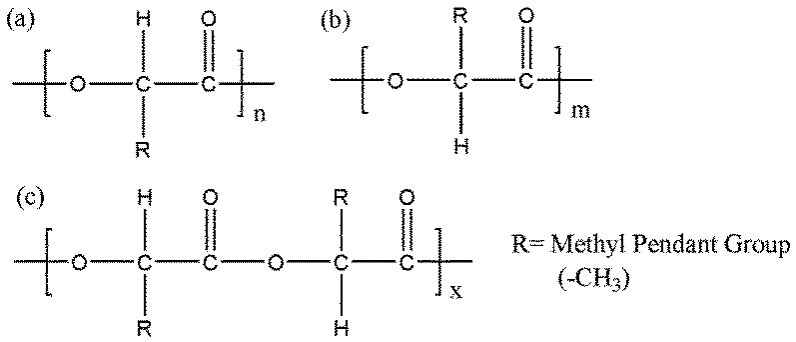


Fig. 2.11: Chemical structure of poly(lactic acid) with (a) D-isomer, (b) L-isomer and (c) DL-isomer

The semicrystalline structure of PLA is provided excellent mechanical strength on PLA's product such as supportive implants and recovery suture. These semicrystalline structures of PLA have induced low degradation rate, which caused PLA taken greater than 2 years to be completely resorbed. In advantages, the degradation rate of PLA microsphere is able to be adjusted from the aspect of molecular weight, crystallinity, particle size and porosity of structure integrity, which have make them attractive to function as a drug delivery system (Nampoothiri *et al.*, 2010, Jiang *et al.*, 2010). In the fabrication of PLA microspheres, it have been reported that the surface morphology of poly(D,L-lactic acid) microsphere is relatively smoother as compared to poly(L-lactic acid) microsphere. This might due to the crystalline and amorphous characteristics (Chung *et al.*, 2001). The molecular weight of PLA gives significant effect on solution's viscosity, which eventually producing larger size microspheres and higher loading efficiency (Benoit *et al.*, 1999).

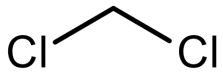
2.4.2 PLA's Solvent

It is important to have a suitable solvent for PLA in microspheres fabrication. Various solvents such as dioxane, acetonitrile, chloroform, methylene chloride, 1,1,2-trichloroethane and dichloroacetatic acid are reported as PLA solvent's. The solubility of PLA is dependent on their characteristic (e.g. molecular weight, stereoisomerism, and crystallinity). In general, amorphous PLA is soluble in most organic solvents, such as tetrahydrofuran, chlorinated solvents, benzene, acetonitrile and dioxane (Garlotta, 2001). While, crystalline poly(L-lactide) is not soluble in acetone, ethyl acetate or tetrahydrofuran, however, it is able to be dissolved at boiling temperature (Nampoothiri *et al.*, 2010).

The general considerations in solvent selection included PLA soluble, poorly soluble in continuous phase, highly volatile with low boiling point, and low toxicity (Li *et al.*, 2008). Dichloromethane (DCM) is commonly used as PLA's solvent in PLA

microspheres fabrication. The properties of DCM are listed in Table 2.4. It contained low boiling point (40˚C) that promise a fast evaporation rate during microspheres precipitation. DCM is a suitable solvent to dissolve semicrystalline PLA at higher dissolution rate, due to their relative small "molar volume" (Auras *et al*., 2010, Jin *et al*., 2010, Abbott, 2010).

Table 2.4: Chemical properties of dichloromethane (Millipore, 2014)

Molecular Structure	Solvent Properties	
	Molar Mass	84.94 g/mol
	Boiling Point	40 ˚C
	Density (20˚C)	1.33 g/cm^3
	Vapour Pressure (20˚C)	475 hPa
	Water Solubility (20˚C)	20 g/L
	Refractive Index	1.42

In polyester-based microspheres fabrication, ethyl acetate has been used to replace DCM as the more biocompatible solvent (Sah, 1997). However, the encapsulation efficiency of microspheres is significantly reduced with the use ethyl acetate (Herrmann and Bodmeier, 1995). Besides, several kind of organic solvents with greater polarity than dichloromethane such as acetone, n-butyl alcohol and ether have been used as replacement solvent or co-solvent in the emulsion system. By using this higher polarity solvent promoting rapid solvent diffusion into the continuous phase, resulting the rapid precipitation microspheres in the system. Zhang *et. al* (2013) reported the used of acetone as co-solvent in DCM have prepared PLA microspheres with higher encapsulation efficiency and absence of emamectin benzoate on microspheres' surfaces. On the other hand, as polymer having high solubility in a solvent, droplet took longer duration to solidify. As the precipitation time increases, the duration of polymer droplets stayed in semi-solid state is extended and resulted higher diffusion of drug into continuous phase. This will reduce the encapsulation efficiency of microspheres (Dhakar *et al*., 2012).